

Published in final edited form as:

Trans R Soc Trop Med Hyg. 2008 December ; 102(12): 1263–1268. doi:10.1016/j.trstmh.2008.06.002.

Prevalence, intensity and risk factor of Clonorchiasis and possible use of questionnaire to detect individuals at risk in northern Vietnam

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Summary

In Vietnam, a high prevalence of liver flukes, *Clonorchis sinensis* and *Opisthorchis viverrini*, has been reported in a number of provinces. Essential knowledge about *C. sinensis* infection gained from Thailand over the past decade suggests a habit of eating raw freshwater fish as a major risk factor of the infection. However, further information to confirm such relationship is needed. In the present study 1,155 villagers in 2 communes in northern Vietnam were interviewed and their stools were examined for presence of liver flukes. The prevalence of the infection was 26% and was 3.6 times higher in males than in females. The habit of eating raw fish increased a risk of *C. sinensis* infection by 53-fold. This article provides evidence of a strong correlation between the intensity of *C. sinensis* infection and the cumulative quantity of freshwater fish consumed in lifetime and suggests that simple questionnaires could be used in endemic areas to quickly identify and treat populations at risk.

Keywords

Liver flukes; *Clonorchis sinensis*; Questionnaire; Vietnam

Introduction

The liver flukes *Clonorchis sinensis* and *Opisthorchis viverrini* cause various hepatobiliary problems and *C. sinensis* is a known risk factor for cholangiocarcinoma, a cancer of the bile ducts. *C. sinensis* and *O. viverrini* are known to have a freshwater snail of the genus *Bithynia*

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Conflicts of interest statement: None declared

Ethical approval: The data presented in this paper was collected during the national Clonorchiasis control activity conducted by the Ministry of Health, Vietnam, in order to improve the performance of the activity. Therefore, ethical approval was not required.

Authors' contributions

TDTC and KNV conceived and designed the study and collected the field data; TDTC administered the questionnaire and conducted molecular analysis; AY and AM carried out the statistical analysis and interpretation of the data and prepared the manuscript; all the authors revised the manuscript and approved the final version of the manuscript. AM is the guarantor of the paper.

as a primary host and a cyprinoid fish species as a secondary host. Human infection occurs when metacercariae in flesh or skin of freshwater fish, which is the infective stage of the liver flukes, are ingested by a human host. Therefore, eating of raw freshwater fish is believed to be the leading risk factor of *C. sinensis* and *O. viverrini* infection. However, the most published descriptions of social habits regarding consumption of raw fish in relation to the infection remains anecdotal (Sithithawarn and Haswell-Elkins, 2003; Lin et al. 2005; Zhang et al. 2007) and additional information to confirm the association between raw fish consumption and the liver fluke infection is in need (Olsen et al., 2006).

World Health Organization (WHO) estimated in 2004 that approximately 1 million people have been infected with *C. sinensis* and *O. viverrini* in Vietnam. Since then, studies have been conducted in a number of communities in Vietnam. According to the extensive review by Nguyen Van De *et al.* (2003), *C. sinensis* is prevalent in northern Vietnam, while *O. viverrini* is predominant in central and southern Vietnam. It reported a generally higher prevalence in males than in females and an increasing prevalence with age.

The objective of the present study was to assess the prevalence and intensity of the liver fluke infection by age, gender and the habit of eating raw fish in Kim Son district, Ninh Binh province in Vietnam.

The study also evaluated the use of questionnaire as a public health tool to identify individuals at risk. This would allow targeting mass drug administration of praziquantel to a smaller group of individuals reducing logistical and drug costs.

Materials and Methods

Location and study participants

The study was conducted in 2 communes (Tan Thanh and Yen Loc) in Kim Son District, Ninh Binh province in Northern Vietnam, which was one of the target areas of national Clonorchiasis control program conducted by the Ministry of Health, Vietnam. The data was collected during the program to reduce the cost for identification of people infected with *C. sinensis*. Ninh Binh is a rural province, about 96 Km to the southeast of Hanoi. The two communes are located on the Red River Delta, about 18 Km from the gulf of Tonkin. The population of Tan Thanh commune and Yen Loc commune is approximately 4,700 and 8,000 people, respectively.

300 households were selected randomly from the name list of the household heads in the two communes. All members of the selected households older than 5 years of age and present on the day of the survey were enrolled in the study.

Molecular genetic techniques

C. sinensis and *O. viverrini* are known to have a very similar morphological feature that is difficult to distinguish from each other (WHO 1994). For differentiation of *C. sinensis* from *O. viverrini*, 315 adult worms were collected from faecal samples of 14 people in Tan Thanh commune, who were given a purgative (Mage Sulphate, Vietnam) a day before. The

collected worms were investigated with molecular genetic characterization using DNA sequence.

The procedure has been described in details elsewhere (Thaenkham *et al.*, 2007; Wongratanacheewin *et al.*, 2002). Briefly, the DNA was extracted from 0.1 gram of adult worms and subjected to PCR amplification. Two sets of primer were used in the study. The first set was CsCoIR2 (GCC TAT AGT GAA AAG CAC CA) and CsCoIF1 (GCA TCC TGA GGT TTA TGT GT), designed using the partial sequences of mitochondrial COI gene of *C. sinensis* and *O. viverrini* (GenBank accession nos. [AF096229](#) and [AY055380](#), respectively). The second set was OV6F (CTG AAT CTC TCG TTT GTT CA) and OV6R (GTT CCA GGT GAG TCT CTC TA), designed using the tandem repetitive sequence of *O. viverrini* of Lao PDR origin (GenBank accession no. [S80278](#)). The second set was used to distinguish *C. sinensis* from *O. viverrini*. The size of the amplified PCR products were 346bp and 330bp, respectively. PCR product was electrophoresised in a 2% agarose gel and photographed under UV light.

Parasitological investigation and questionnaire

Each study participants received a plastic container and was requested to collect a single stool sample of around 10 gram and to return it to the commune health station the following day. Upon the returning of the containers, each study participant was interviewed using the simple questionnaire about gender, age and the habit of eating raw fish. Those who reported raw fish consumption were also asked about the time since when the habit started, the frequency and the amount of raw fish consumed in each event.

Stool samples were examined by Kato-Katz thick smear method (Katz et al., 1972) with template size of 41.7mg and multiplication factor of 24. The number of *C. sinensis*/*O. viverrini* eggs present in each sample was recorded.

Informed consent was obtained from each individual participant to the study before enrolment.

Data analysis

Results of fecal examination and questionnaire survey were entered in Excel and statically analyzed using EpiInfo 3.3.2 (CDC, Atlanta, GA, USA) for prevalence and intensity of infection (epg) as measured by enumeration of eggs per gram feces. The study participants were subdivided into seven age groups: (1) <10 years; (2) 10-19 years; (3) 20-29 years; (4) 30-39 years; (5) 40-49 years; (6) 50-59 years; (7) >59 years. The univariate analysis of the relationship between the prevalence and the risk factors, namely gender, age groups, and habit of eating raw fish, was conducted. The risk factors showing a significant relationship with the prevalence were then subjected to logistic regression analysis. To examine univariate association between the intensity of infection and risk factors, geometric mean epg (logarithmic transformation of $\text{epg}+1$) were statistically analyzed by Mann-Whitney/Wilcoxon test (for gender and raw fish consumption) and Spearman's rank correlation (for age groups). The factors that showed a significant association with the intensity were then subjected to multiple regression analysis to eliminate confounding factors. The "cumulative

quantity of raw fish consumed" by each individual (during his lifetime) was calculated multiplying the years of raw fish consumption by the mean number of occasions in which raw fish was consumed each year and the quantity of raw fish consumed per occasion. The means of the above 4 variables stratified by the level of the intensity (<1000epg, 1000 – 5000epg, > 5000epg) was calculated and subjected to chi-square test for analysis of associations.

Results

PCR Identification of *C. sinensis*

Amplified PCR products of all the samples generated a PCR fragment of 346bp. On the other hand, the fragment of the tandem repetitive sequence of *O. viverrini*, was not amplified from any of the samples. This result indicated that the liver flukes collected from the fecal samples of villagers in Tan Thanh commune, Kim Son district, Ninh Binh province were not *O. viverrini* but identical to *C. sinensis* originating from Lao PDR.

Prevalence of infection

Stool samples were obtained from 1,155 individuals from 2 to 79 years of age. In the sample were present 97 children below 9 years of age (8.4%). The sample was constituted by 33.9% of females. The prevalence of *C. sinensis* infection in the sample was 26.1% with significant difference between males (33.8%) and female (11%) ($p<0.01$).

Figure 1 illustrates the prevalence of *C. sinensis* infection by age and gender. None of the children below 9 years of age were infected with *C. sinensis*. The youngest age of the infection was found to be 13, while the oldest age was 75 years old. The prevalence increased with age and reached a plateau in the 40 – 49 years age group. In the all age groups, infection rates were significantly higher in males than in females ($p<0.01$ for all age groups).

Intensity of infection

The average counts of *C. sinensis* eggs among 301 positive samples were 472 eggs per gram of feces (epg). The highest count was 15,801 eggs per gram feces found in a male sample at 55 years of age. Mann-Whitney/Wilcoxon test applied on the log-transformed mean egg counts showed significant difference in infection intensity between males and females ($p<0.001$), among different age groups ($p<0.001$) and between those eating raw fish and those not ($p<0.001$). According to multiple regression analysis, however, habit of eating raw fish was found to be the main factor associated with increased intensity ($p<0.001$) (Table 2). Figure 2 illustrates the arithmetic mean intensity of *C. sinensis* infection by age and gender. The intensity of *C. sinensis* infection in male population increased with age and reached its peak in the 50 – 59 years age group. On the other hand, there was no linear relationship between age and the infection intensity in female population. The peaks of the intensity in females were observed in 10 – 19 years group (391 epg) and 50 – 59 years group (1189 epg), which were even higher than those of the male population.

Quantity of raw fish consumption and *C. sinensis* infection

Consumption of raw fish was reported by 75.1% of the study population between 20 and 60 years of age (863 individuals). None of the study participants below 20 years of age reported eating raw fish. The number of males having the habit of raw fish consumption was 3 times higher than that of females.

Correlation of infection with the positive response to the questionnaire

Table 1 summarizes results of logistic regression to analyze the association between the prevalence of *C. sinensis* infection and report of consumption of raw fish as well as age and gender of the respondents.

The positive answer to the question about the habit of eating raw fish was found to correspond to an increased risk of infection by 53-fold (95% CI 19.94-161.67), while gender and age were confounding factors. While approximately half of the people reporting eating raw fish have been infected (44.6%), only 3.3 % of those not reporting a habit of eating raw fish were found to be infected with *C. sinensis*. Habit of eating raw fish was also found to be the main factor of increased infection intensity. Sensitivity, specificity, positive and negative predictive values of screening by raw fish consumption was 0.98, 0.37, 0.45 and 0.97, respectively.

Table 3 summarizes information on cumulative quantity of raw fish consumption obtained from 159 study participants who reported raw fish consumption and were also infected with *C. sinensis*. The intensity of the infection illustrated a positive association with history of raw fish consumption ($\chi^2=23.2$, $p<0.01$), annual frequency of raw fish consumption ($\chi^2=44.7$, $p<0.01$), quantity of raw fish consumed per occasion ($\chi^2=23.7$, $p<0.01$) and the cumulative quantity of raw fish reported to be consumed by the time of the survey ($\chi^2=73.8$, $p<0.01$).

Discussion

The high level of *C. sinensis* infection documented in this study confirms the previous findings by Nguyen Van De et al. (1997), Kino et al. (1998), Le Van Chau et al. (2001), Kieu Tung Lam et al. (1992) and Nguyen Thi Hung (1999) in the same province. The study also confirmed the report of Nguyen Van De et al (2003) concerning the fact that *C. sinensis* is the food borne trematode specie more prevalent in northern Vietnam.

The prevalence of infection was higher in males than in females, and it increased with age in both genders. Those findings are consistent with previous reports from Vietnam as well as China, Thailand and Laos (Nontasut et al. 2003; Kieu Tung Lam et al. 1992; Chen et al. 1994; Sornmani et al., 1973; Sayasone et al., 2007; Zhang et al., 2007; Lin et al., 2005) and it is probably due to the more frequent raw fish eating in males (Nontasut et al., 2003). In our study population, males had 3-fold more occasions of raw fish consumption than females.

The 3 % *C. sinensis* infection of the people not reporting raw fish eating is probably due to under-report or a possibility of cross-contamination during cooking process as metacercariae

of *C. sinensis* is mucilaginous and possibly sticks to cooking utensils and could theoretically contaminate other food. The concern on using the same utensils for raw fish and cooked food has been discussed by Zhang et al (2007).

The progressive increase of prevalence and intensity of infection with age suggests a possible accumulation of *C. sinensis* infection over time by re-infection through repetitive consumption of raw fish in male population. The irregular pattern of the infection intensity by age in females in our study might indicate that many of the infected females are sporadically consuming raw fish or ingesting the metacercariae through cross-contamination rather than practicing a regular eating of raw fish as males commonly do.

Repetitive infection with *C. sinensis* is known to increase a risk of cholangiocarcinoma, a cancer of the bile duct (Parkin et al., 1993). While no reports on the prevalence of cholangiocarcinoma in Vietnam have been published so far, a high prevalence of cholangiocarcinoma and its clear association with the liver fluke infection have been increasingly revealed in Thailand (Wiwanitkit 2003; Parkin et al., 2006). This demonstrates the need for further researches on the prevalence of cholangiocarcinoma in the communities in Vietnam where a high prevalence of *C. sinensis* infection has been identified.

The strong association to the report in questionnaire of raw fish consumption to the prevalence and the intensity of *C. sinensis* infection in our opinion strongly suggest that, similarly to questionnaires used for the identification of schistosomiasis (WHO 1995), simple questionnaires investigating food habits could be used to screen population in areas where clonorchiasis is transmitted permitting to apply large scale chemotherapy with praziquantel avoiding the cost of the parasitological investigation. Finally, an importance of promoting hygienic diets and food processing was realized for prevention of *C. sinensis* infection.

Acknowledgements

We are indebted to the staffs of the National Institute of Veterinary Research (Hanoi) and the National Institute of Malariology, Parasitology and Entomology (Hanoi) for technical assistance. We also thank the active corporation of the authorities of the Department of Health on provincial, district and commune level as well as the people of Kim Son district.

Funding: None

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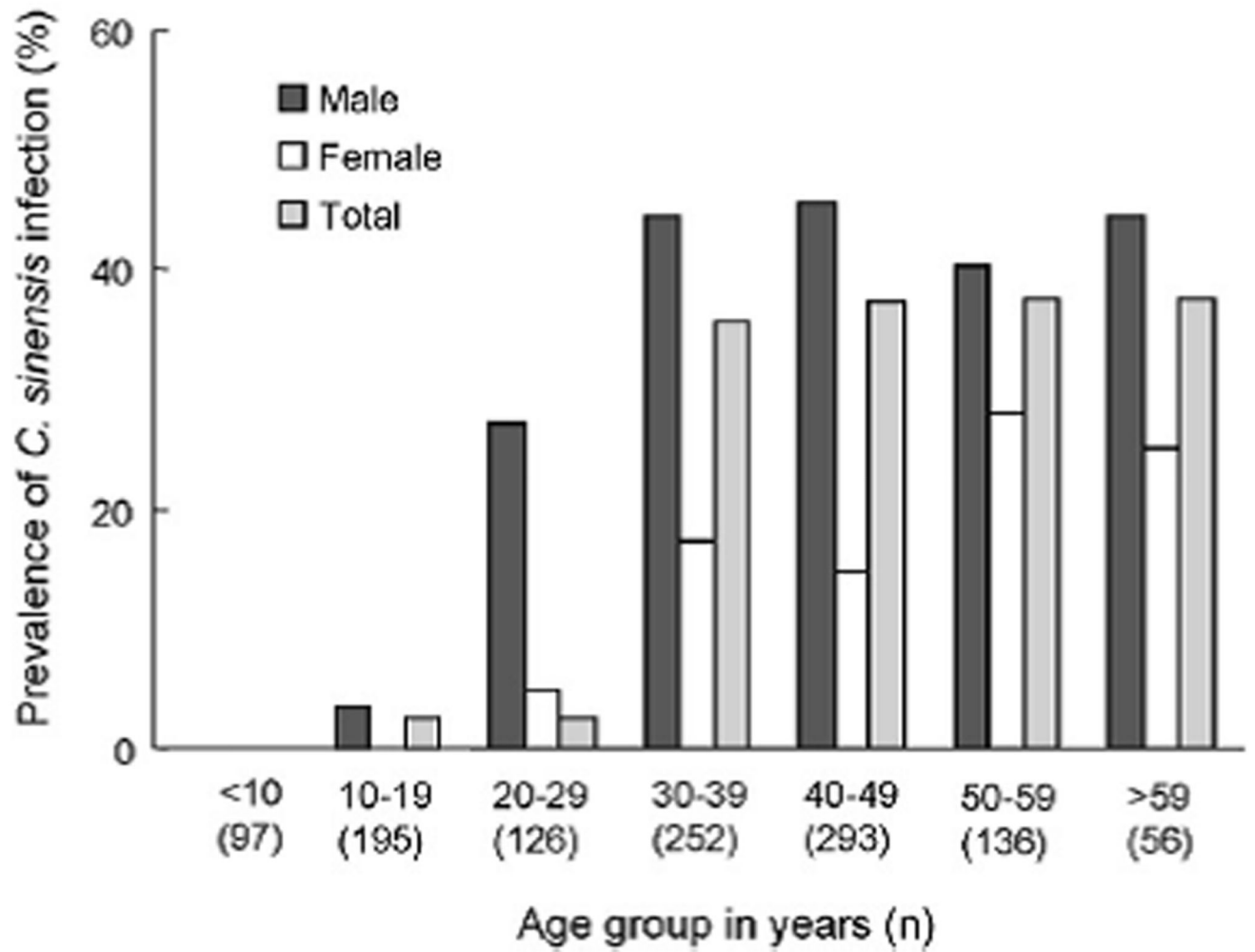


Figure 1. Prevalence of *C. sinensis* infection by age and gender in 2 commune, Kin Som district, Ninh Binh province, Vietnam, 1999-2000 (n=1155).

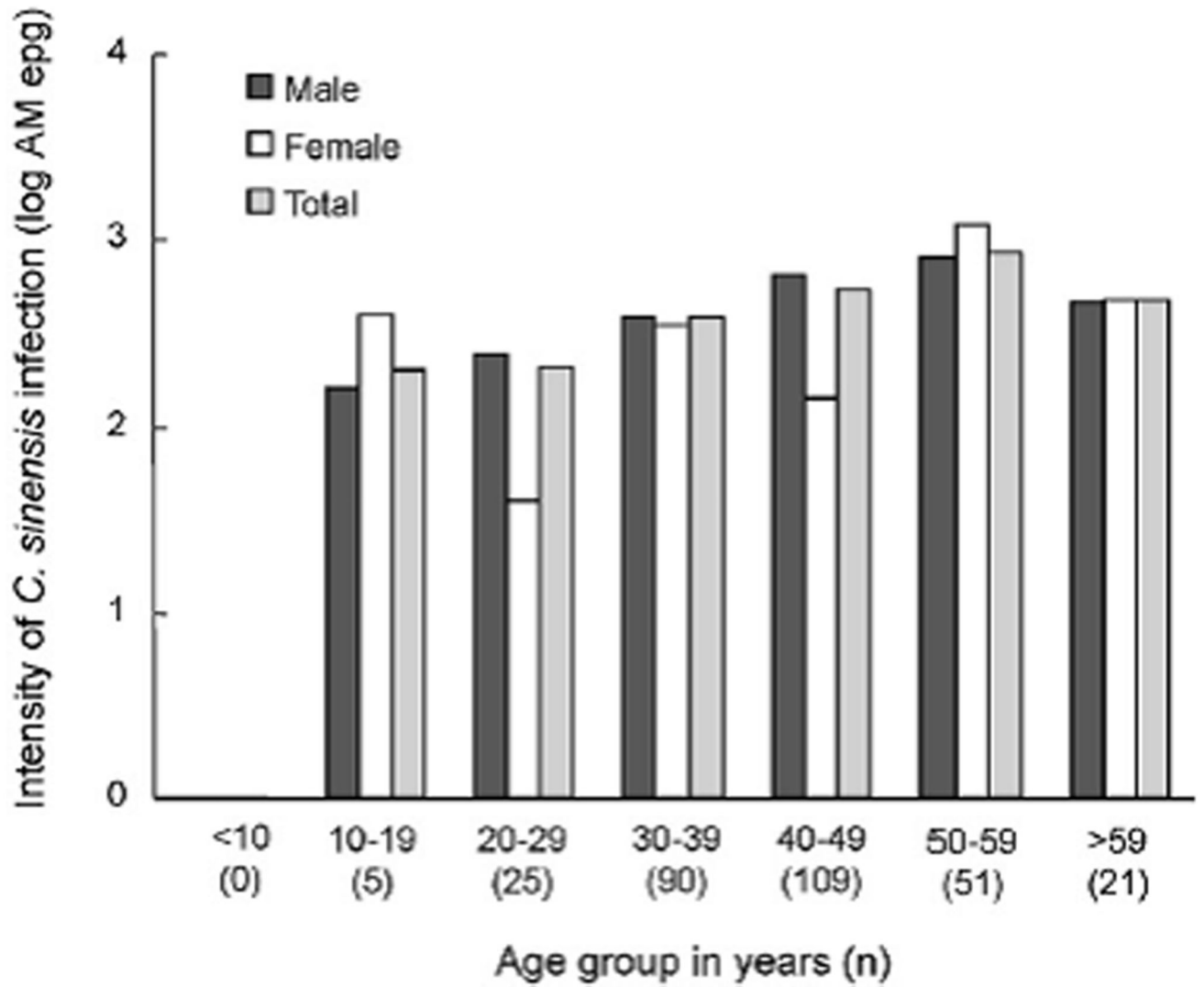


Figure 2.
Intensity of *C. sinensis* infection by age and gender in 2 commune, Kin Som district, Ninh Binh province, Vietnam, 1999-2000 (n=301).

Table 1

Crude and adjusted odds ratio and 95% confidence intervals (CI) for three risk factors relative to *C. sinensis* infection in 2 commune, Kin Som district, Ninh Binh province, Vietnam, 1999-2000 (n=1155).

Variable	n	Mean (range) epg ^a	P-value (unadjusted) ^b	P-value (adjusted) ^c
Gender				
Male	258	461.32 (0–15801)	<0.001	0.99
Female	43	138.19 (0–7797)		
Age (years)				
<10	0		<0.001	0.66
10–19	5	196.45 (0–391)		
20–29	25	205.03 (0–2047)		
30–39	90	375.45 (0–7245)		
40–49	109	544.76 (0–9085)		
50–59	51	863.33 (0–15 801)		
>59	21	468.18 (0–7797)		
Habit of eating raw fish				
Yes	289	497.17 (0–15 801)	<0.001	<0.001
No	7	33.64 (0–2553)		

^aArithmetic mean (range) egg counts per gram feces (epg).

^bP-values from univariate analysis for each risk factor.

^cP-values from multiple regression analysis for all the three risk factors.

Table 2

General linear model for factors associated with the intensity of *C. sinensis* infection in 2 commune, Kin Som district, Ninh Binh province, Vietnam, 1999-2000 (n=301).

Variable	N	Prevalence n (%)	Crude odds ratio (95% CI)	P-value (unadjusted) ^a	Adjusted odds ratio (95% CI) ^b	P-value (adjusted) ^c
Gender						
Male	764	258 (33.8)	4.12 (2.91–5.86)	<0.001	0.67 (0.41–1.10)	0.11
Female	391	43 (11.0)				
Age (years)						
<29	418	30 (7.2)				
>29	737	271 (36.8)	7.52 (5.04–11.23)	<0.001	1.07 (0.62–0.27)	0.82
Habit of eating raw fish						
Yes	648	289 (44.6)	57.50 (26.84–123.18)	<0.001	53.15 (19.94–161.67)	<0.001
No	507	7 (3.3)				

^a P-values from univariate analysis for each risk factor.

^b Odds ratios from logistic regression analysis for all the three risk factors.

^c P-values from logistic regression analysis for all the three risk factors.

Table 3

Summary of the association between the intensity of *C. sinensis* infection and the cumulative quantity of raw fish consumed in each interviewee's history by the time of the survey in 2 commune, Kin Som district, Ninh Binh province, Vietnam, 1999-2000 (n=159).

Variable	n	Mean epg±SD (x10 ³)
History of raw fish consumption (years) ^a		
<10	71	0.84 ± 0.67
10–20	62	1.51 ± 1.46
20–30	25	2.62 ± 3.56
>30	1	0.62 ± 0
Annual frequency of raw fish consumption (times/year)		
<10	56	0.56 ± 0.25
10–20	65	1.19 ± 1.09
21–40	38	2.93 ± 2.92
Quantity of raw fish consumed per occasion (g)		
<100	74	0.62 ± 0.37
100–200	63	1.55 ± 1.50
201–400	8	3.94 ± 3.70
>400	14	1.38 ± 1.83
Cumulative quantity of raw fish consumed in lifetime (g) ^b		
<1000	45	0.41 ± 0.20
1000–2000	33	0.79 ± 0.24
2001–4000	28	0.97 ± 0.35
4001–8000	25	1.58 ± 0.84
8001–16000	15	3.50 ± 2.05
>16000	13	4.51 ± 4.03

^aHistory of raw fish consumption by the time of the study, expressed in years.

^bCumulative quantity of raw fish consumed in lifetime by the time of the study.